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### **NCAM is at the heart of reciprocal regulation of E-cadherin- and integrin-mediated adhesions via signaling modulation**

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distribution of labeled visceral endoderm cells to see how they become replaced by the invading epiblast-derived endoderm. All three methods give the same result: epiblast-derived endoderm cells insert into the endodermal layer as single cells, which is confirmed very clearly by time-lapse movies. Surprisingly, almost all of the visceral endoderm cells appear to persist and even to proliferate, although they become gradually diluted by the newcomers. It will be interesting in future to determine whether, as in the chick, prospective mouse endoderm cells travel within a middle (mesendoderm) layer before joining the endoderm as single cells.

Another surprising observation is that the persisting visceral endoderm cells can be detected as late as the 16–18 somite stage in the lining of the gut, indicat-

ing that these cells do contribute to the embryo proper. Furthermore, these cells tend to surround embryonic structures that have known signaling properties, such as the node and head process/notochord (Figures 1B and 1C). The authors are rightly cautious to avoid speculating on the significance of this, but no doubt others will soon propose that their continued presence around these structures could explain some of the signaling functions of these centers. One hopes that any such proposals will be accompanied by evidence as compelling as that in the present study, which is a model of how shrewd observations, untainted by preconceptions and supported by several very well-designed embryological experiments, can reveal that even the most widely accepted “facts” can sometimes be wrong.

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# NCAM Is at the Heart of Reciprocal Regulation of E-Cadherin- and Integrin-Mediated Adhesions via Signaling Modulation

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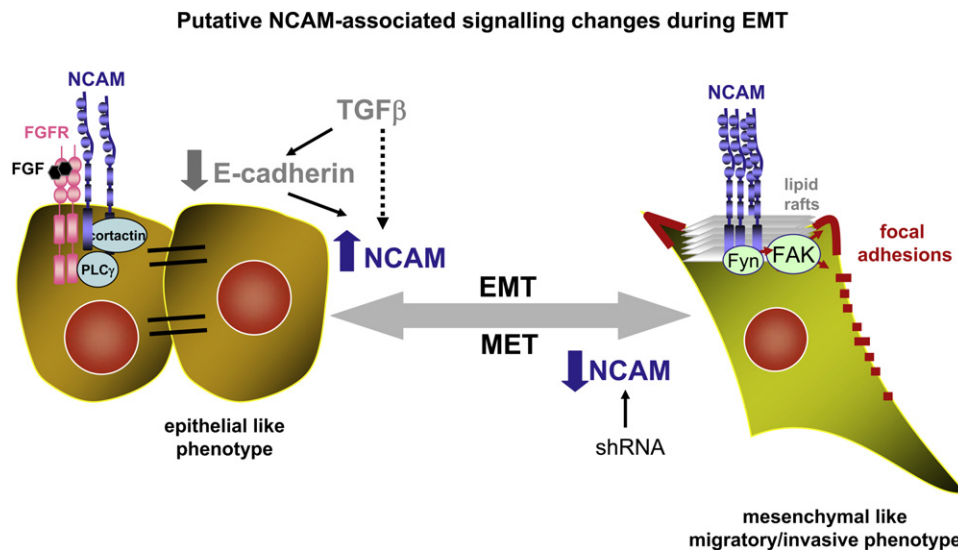
**New work by Lehenbre et al. in *The EMBO Journal* reveals that the cell-adhesion molecule, NCAM, is at the heart of crosstalk between E-cadherin loss and reciprocal focal adhesion assembly during the epithelial to mesenchymal transition (EMT). NCAM upregulation induces the formation of novel signaling complexes that correlate with NCAM-dependent focal adhesion assembly, migration, and cancer cell invasion.**

E-cadherin is a multitissue tumor-suppressor protein which is often lost, or dysfunctional, in epithelial cancers (Cavallaro and Christofori, 2004). As a consequence, cell-cell adhesions (adherens junctions) are weakened, and this weakening facilitates cells' breaking free from their neighbors. In addition, cells assemble dynamic  $\beta$ 1-integrin-mediated focal adhesions or focal contacts and assume a migratory phenotype more reminiscent of mesenchymal cells. Epithelial to mesenchymal

transition (EMT) occurs during embryonic development and is presumed to play a role in acquisition of invasive and malignant phenotype (Lee et al., 2006; Yang and Weinberg, 2008). Moreover, TGF $\beta$  (which is used extensively in the new study by Lehenbre et al. [2008] in *The EMBO Journal*) promotes EMT by Smad-mediated transcriptional activation of HMGA2, which induces expression of Snail, Twist, and Slug that, in turn, repress E-cadherin (Thuault et al., 2006). The im-

portance of EMT during normal development and pathophysiological processes has justifiably led to its intense investigation over many years. However, we still lack a full understanding of the critical mediators of EMT initiation and maintenance and of the mechanisms involved in reciprocal regulation of E-cadherin and the components of integrin-mediated focal adhesions.

In a new study, several experimental cell systems, transgenic mouse models,



**Figure 1.**

Induction of EMT, induced via TGF $\beta$  or cadherin loss, not only causes NCAM-mediated weakening of cell-cell adhesions, but also formation and dynamic turnover of focal adhesions. This is accompanied by elevated NCAM expression, which leads to altered signaling complexes. Specifically, NCAM binding to PLC $\gamma$  and cortactin is diminished, and NCAM forms a complex with Fyn (and likely FAK downstream). The induced complexes sediment in detergent insoluble membrane fractions of sucrose gradients, implying that these are in lipid rafts. These signaling changes are linked to induction of the more mesenchymal and migratory phenotype associated with aggressive cancers of epithelial origin. Knockdown of NCAM induces the reversal of EMT (i.e., MET [mesenchymal to epithelial transition]).

and tumor material, are used to address thoroughly the role of the Ig domain homotypic adhesion protein, NCAM, in promoting EMT (Lehembre et al., 2008). Intervention strategies clearly establish that expression of NCAM is commonly upregulated and promotes an adhesion switch during EMT that is associated with cancer invasion. In particular, TGF $\beta$  treatment or diminished E-cadherin function induces changes in adhesion protein expression that is typical of either “cadherin switching” (i.e., reduced E-cadherin and elevated N-cadherin) or full EMT, during which vimentin becomes expressed. In both cases, NCAM is induced to a greater or lesser extent. Although TGF $\beta$  acts via Smads to exert this effect, the precise mechanism by which NCAM promoter activity is regulated by E-cadherin loss or by TGF $\beta$ -induced Smad activity remains to be established.

NCAM is not only needed for induction of EMT but also for maintenance of the mesenchymal state. In keeping with a more general role, enforced expression of NCAM promotes mesenchymal-like properties in some epithelial cells in culture. For example, there is reciprocal staining of E-cadherin and NCAM in tumor sections from the RipTag2 mouse model of pancreatic cancer, and NCAM defi-

ciency leads to aberrant persistence of E-cadherin expression. The reciprocal regulation of E-cadherin and NCAM in tumors in vivo may explain why reduced NCAM expression promotes tumor dissemination (Perl et al., 1999). In fact, analogous results are seen using a mouse model of lobular breast carcinogenesis (Derksen et al., 2006) and human patient samples, where the reciprocal expression of NCAM and E-cadherin correlate strongly with invasive and well-differentiated phenotypes, respectively. These findings imply that the causal role of NCAM during EMT is indeed relevant to human cancer.

However, the role of NCAM is not limited to its relationship to E-cadherin. Perhaps the most striking findings of the new study are that NCAM has a very clear role in assembly of  $\beta$ 1-integrin-dependent focal adhesions in cells that retain epithelial-mesenchymal plasticity, and that this is accompanied by changes in NCAM-associated signaling complexes and redistribution to distinct membrane microdomains. NCAM induces FAK phosphorylation and enhanced  $\beta$ 1-integrin-dependent cell spreading, while NCAM-expressing MDCK cells scatter at low density and become extruded from mixed monolayers, migrating on top of their epi-

thelial neighbors that do not overexpress NCAM at high density. However, NCAM knockdown causes NMuMG cells to migrate faster in both scratch wound and Boyden chamber assays, suggesting that both assembly and dynamic turnover of cell-matrix adhesions are influenced by NCAM.

A switch between two distinct, apparently mutually exclusive, signaling complexes may provide the mechanism by which NCAM regulates focal adhesion assembly and turnover. Under normal conditions, NCAM associates with FGFR, PLC $\gamma$ , and cortactin and sediments in detergent-soluble membrane fractions in sucrose gradients. Upon TGF $\beta$  treatment, NCAM no longer associates with PLC $\gamma$  or cortactin, instead associating with Fyn, whose tyrosine phosphorylation is enhanced. Moreover, a proportion of NCAM, Fyn, and FAK now cosediment in detergent-insoluble membrane fractions, suggesting that NCAM switches binding partners and associates with the adhesion-linked tyrosine kinases Fyn, and perhaps also FAK, in lipid rafts (Figure 1). These findings raise a number of interesting questions. For example, does NCAM associate solely with Fyn, or are other Src family kinases (Src and/or Yes) involved, and is FAK also present in the

same complex at lipid rafts? Molecular intervention is needed to establish whether formation of the proposed NCAM/Fyn/FAK complex at lipid rafts mediates focal adhesion assembly, and turnover, during EMT. The authors noted that the NCAM-FGFR complex persists after TGF $\beta$  treatment, and it would be interesting to determine whether or not a proportion of FGFR is also present in lipid rafts. It had been shown previously that NCAM forms a complex with Fyn and FAK in lipid rafts upon homodimerization in neurons (Beggs et al., 1997); the new study suggests this complex may also a role in mediating NCAM-dependent events during EMT. Whether FAK, or other integrin effectors, not only influences focal adhesion assembly but also feeds back on E-cadherin dynamics or stability at the membrane is an intriguing possibility.

The localized expression of NCAM almost exclusively at invasive tumors margins may have precluded the inclusion of NCAM in "signatures" associated with poor prognosis, as derived from large-scale microarray analyses. However, tis-

sue microarray analysis may reveal the potential prognostic significance of NCAM expression. The current study highlights the undoubted importance of NCAM in promoting signaling changes at specific membrane microdomains, and in inducing both focal adhesion formation and E-cadherin loss during EMT. Consequently, NCAM has a central role in promoting one set of dynamic adhesion complexes (focal adhesions), apparently at the expense of another (adherens junctions), and so inducing migratory properties associated with aggressive cancer phenotypes. NCAM, and perhaps other related Ig domain-containing adhesion proteins, may prove to be useful as early markers of EMT in vivo. Early changes associated with EMT are difficult to visualize morphologically in tumor sections, yet early detection of EMT may predict likely invasive behavior and could aid decisions about appropriate treatment. Also, there is substantial interest now in anti-EMT therapeutic strategies, which will require good biomarkers of early changes during EMT to monitor efficacy.

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